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(57) Abstract

This invention relates to a method of immunizing a vertebrate, comprising introducing into the vertebrate a DNA transcription unit which comprises DNA encoding a desired antigen or antigens. The uptake of the DNA transcription unit by a host vertebrate results in the expression of the desired antigen or antigens, thereby eliciting humoral or cell-mediated immune responses or both humoral and cell-mediated responses. The elicited humoral and cell-mediated response can provide protection against infection by pathogenic agents, provide an anti-tumor response, or provide contraception. The host can be any vertebrate, avian or mammal, including humans.

IMMUNIZATION BY INOCULATION OF DNA TRANSCRIPTION UNIT

Background of the Invention

Vaccination with inactivated or attenuated organisms or their products has been shown to be an effective method for increasing host resistance and ultimately has led to the eradication of certain common and serious infectious diseases. The use of vaccines is based on the stimulation of specific immune responses within a host or the transfer of preformed antibodies. The prevention of certain diseases, such as poliomyelitis, by vaccines represents one of immunology's greatest triumphs.

Effective vaccines have been developed for relatively few of the infectious agents that cause disease in domestic animals and man. This reflects technical problems associated with the growth and attenuation of virulent strains of pathogens. Recently effort has been placed on the development of subunit vaccines (vaccines that present only selected antigens from a pathogen to the host). Subunit vaccines have the potential for achieving high levels of protection in the virtual absence of side effects. Subunit vaccines also offer the opportunity for the development of vaccines that are stable, easy to administer, and sufficiently cost-effective for widespread distribution.

Summary of the Invention

This invention relates to a method of subunit vaccination. Specifically, this invention relates to a method of immunizing an individual, comprising introducing into the individual a DNA transcription unit (or units) which comprises DNA encoding a desired antigen or antigens and DNA encoging a transcriptional promoter element or

elements. A single transcription unit or multiple DNA transcription units can be administered to an individual to achieve immunization against one antigen or multiple antigens. The uptake of the DNA transcription units by host cells results in the expression of the desired antigen or antigens, thereby eliciting humoral or cell-mediated immune responses or both humoral and cell-mediated responses. The elicited humoral and cell-mediated immune response can provide protection against infection by pathogenic agents, provide an anti-tumor response, or provide contraception. The host can be any vertebrate, avian or mammalian, including humans.

The present invention relates to the use of DNA transcription units for raising immune responses. In one embodiment, the individual is immunized by parenteral routes of inoculation. These include intravenous, intramuscular, intradermal, and subcutaneous administration of DNA transcription units. DNAs administered to the skin can be delivered with a DNA gun. In a second embodiment, the individual is immunized by contacting a mucosal surface, such as a respiratory mucosal surface, with DNA transcription units in such a manner that the transcription units are taken up by (i.e., enter the cells of) the mucosal surface. DNAs for mucosal administration can be microsphere encapsulated.

The DNA transcription units introduced by the present method can be used to express any antigen encoded by an infectious agent, such as a virus, a bacterium, a fungus, or a parasite, as well as antigenic fragments and peptides that have been experimentally determined to be effective in immunizing an individual against infection by a pathogenic agent. As stated above, DNA transcription units can also be used for contraceptive purposes or for anti-cancer therapy.

The desired antigens to be expressed can be designed so as to give internal, surface, secreted, or budding and assembled forms of the antigens being used as immunogens.

There are numerous advantages of the use of DNA for immunizations. For example, immunization can be accomplished for any antigen encoded by DNA. Furthermore, the DNA encoded antigens are expressed as "pure" antigens in their native states and have undergone normal host cell modifications. Also, DNA is easily and inexpensively manipulated and is stable as a dry product or in solution over a wide range of temperatures. Thus, this technology is valuable for the development of highly effective subunit vaccines.

Brief Description of the Drawings

Figure 1 is a schematic representation of a bacterial plasmid containing a DNA transcription unit (referred to as pP1/H7) comprising an influenza virus hemagglutinin type 7 (H7) gene expressed by a replication competent retroviral vector.

Figure 2 is a schematic representation of a bacterial plasmid containing a DNA transcription unit (p188) comprising an influenza virus hemagglutinin type 7 (H7) gene expressed by a replication defective retroviral vector.

Figure 3 is a schematic representation of a bacterial plasmid comprising a retroviral vector (pRCAS) with no H7 insert, used as a control.

Figure 4A is a schematic representation of the nonretroviral vector comprising the influenza virus antigen DNA transcription unit encoding subtype H7 hemagglutinin.

CLAIMS

1. Use of a DNA transcription unit comprising DNA encoding a desired antigen operatively linked to DNA which is a promoter region, for the manufacture of a medicament for use in vertebrate immunization by eliciting a protective immune response against a rotavirus or an immunodeficiency virus, wherein the protective immune response is a humoral immune response and/or a cell-mediated immune response elicited against the desired antigen.

2. A method of immunizing a vertebrate by eliciting a protective immune response against a rotavirus or an immunodeficiency virus, said method comprising administering to a vertebrate a DNA transcription unit comprising DNA encoding a desired antigen operatively linked to DNA which is a promoter region, whereby the protective immune response is a humoral immune response and/or a cell-mediated immune response elicited against the desired antigen.

3. A product comprising a DNA transcription unit, which transcription unit comprises DNA encoding a desired antigen operatively linked to DNA which is a promoter region, wherein the DNA is microsphere encapsulated.

4. A product comprising a microsphere encapsulated DNA transcription unit, which transcription unit comprises DNA encoding a desired antigen operatively linked to DNA which is a promoter region, for use in therapy e.g. for use in vertebrate immunization by eliciting a humoral immune response and/or a cell-mediated immune response elicited against the desired antigen.

5. Use of a microsphere encapsulated DNA transcription unit, which transcription unit comprises DNA encoding a desired antigen operatively linked to DNA which is a promoter region, for the manufacture of a medicament for use in vertebrate immunization by eliciting a humoral immune response and/or a cell-mediated immune response elicited against the desired antigen.

6. A method of immunizing a vertebrate, said method comprising administering to a vertebrate a microsphere encapsulated DNA transcription unit, the DNA transcription unit comprising DNA encoding a desired antigen operatively linked to DNA which is a promoter region, whereby a humoral immune response and/or a cell-mediated immune response is/or elicited against the desired antigen.

7. A product comprising more than one DNA transcription unit, each transcription unit comprising DNA encoding a desired antigen operatively linked to DNA which is a promoter region, whereby a humoral immune response ad/or a cell-mediated immune response is/are elicited against the desired antigen, wherein the desired antigen for one transcription unit is different from the desired antigen of the or each of the other transcription unit(s).

8. A product for use in therapy comprising more than one DNA transcription unit, each transcription unit comprising DNA encoding a desired antigen operatively linked to DNA which is a promoter region, wherein the desired antigen for one transcription unit is different from the desired antigen of the or each of the other transcription unit(s), e.g. for use in a

method of immunizing a vertebrate by administering the product whereby a humoral immune response and/or a cell-mediated immune response is/are elicited against the desired antigen.

9. Use of a product comprising more than one DNA transcription unit for the manufacture of a medicament for immunizing a vertebrate by administering to said vertebrate the product, wherein each transcription unit comprises DNA encoding a desired antigen operatively linked to DNA which is a promoter region, whereby a humoral immune response and/or a cell-mediated immune response is/are elicited against the desired antigen, wherein the desired antigen for one transcription unit is different from the desired antigen of the or each of the other transcription unit(s).

10. A method of immunizing a vertebrate, said method comprising administering to a vertebrate more than one DNA transcription unit, each transcription unit comprising DNA encoding a desired antigen operatively linked to DNA which is a promoter region, whereby a humoral immune response and/or a cell-mediated immune response is/are elicited against the desired antigen, wherein the desired antigen for one transcription unit is different from each of the desired antigen of each of the other transcription units.

11. A product, use or method according to any one of claims 7, 8, 9 or 10, wherein the different antigens elicit a protective response from an influenza virus.

12. A product, use or method according to claim 11, wherein the different antigens are from different subtypes of influenza.

13. A product, use or method according to claim 11, wherein the different antigens are from different subgroups of influenza.

14. A product, use or method according to claim 11, wherein the different antigens are from different subtypes of influenza and from different subgroups of influenza.

15. A product, use or method according to any one of claims 7, 8, 9 or 10, wherein the different antigens elicit a protective response from an immunodeficiency virus.

16. A product, use or method according to claim 15, wherein the different antigens represent different subgroups of the immunodeficiency virus.

17. A product, use or method according to claim 15, wherein the different antigens represent different phases of infection of the immunodeficiency virus.

18. A product, use or method according to claim 15, wherein the different antigens represent different tissue tropisms of the immunodeficiency virus.

19. A product, use or method according to claim 15, wherein the different antigens represent different routes of transmission of the immunodeficiency virus.

20. The product, use or method according to any one of claims 1, 2, or 7 to 19, wherein the transcription unit is microsphere encapsulated.

21. The product, use or method according to any one of claims 1 to 6, wherein more than one transcription unit is administered to the vertebrate, wherein the desired antigen for one transcription unit is different from the desired antigen of the or each of the other transcription unit(s).

22. The product, use or method according to claim 21 further including the features of any one of claims 11 to 19.

23. The product, use or method according to any one of the preceding claims for administration to a vertebrate through a route of administration selected from the group consisting of intravenous, intramuscular, intraperitoneal, intradermal and subcutaneous.

24. The product, use or method according to any one of the claims 1 to 22 for administration to a vertebrate by contact to a mucosal surface.

25. The product, use or method according to claim 24, wherein the mucosal surface is a respiratory mucosal surface, such as a nasal mucosal surface or a tracheal mucosal surface.

26. The product, use or method according to any one of the preceding claims, wherein the promoter region of the transcription unit is either of retroviral origin or non-retroviral origin.

27. The product, use or method according to any one of the preceding claims, wherein the vertebrate is a mammal, such as a human.

28. The product, use or method according to any one of the preceding claims, wherein the transcription unit is directly expressed by host cell factors.

29. The product, use or method of any one of the preceding claims, wherein the desired antigen is capable of eliciting a protective immune response against a virus.

30. The product, use or method of claim 29, wherein the virus is an influenza virus.

31. The product, use or method of claim 30, wherein the desired antigen is an influenza virus hemagglutinin.

32. The product, use or method of claim 29, wherein the virus is a human immunodeficiency virus.